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Brain-derived neurotrophic factor and autism: maternal and infant peripheral blood levels in the Early Markers for Autism (EMA) Study

Lisa A. Croen[1], **Paula Goines**[2], **Daniel Braunschweig**[2], **Robert Yolken**[3], **Cathleen K. Yoshida**[1], **Judith K. Grether**[4], **Bruce Fireman**[1], **Martin Kharrazi**[5], **Robin Hansen**[6], and **Judy Van de Water**[2]

[1] *Division of Research, Kaiser Permanente Northern California, Oakland, California* [2] *Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis* [3] *Division of Developmental Neurovirology, Johns Hopkins University, Baltimore, Maryland* [4] *Environmental Health Investigations Branch, California Department of Public Health, Richmond, California* [5] *Genetics Disease Screening Program, California Department of Public Health, Richmond, California* [6] *M.I.N.D. Institute, University of California at Davis*

Abstract

LAY ABSTRACT—The diagnosis of autism is based solely on behavioral characteristics. There is currently no laboratory test that can be done to identify autism. In this study, we investigated a molecule called brain derived neurotrophic factor (BDNF) as a possible early biologic marker for autism. BDNF is a small protein found throughout the central nervous system and in circulating blood. We measured the level of BDNF in blood collected from women during pregnancy and from their babies at birth. We found that the concentration of BDNF in the maternal mid-pregnancy and newborn blood specimens was similar for children with autism, children with mental retardation, and children with typical development. The results of this study suggest that BDNF is unlikely to be a useful early biologic marker for autism.

Objective: To investigate levels of brain-derived neurotrophic factor (BDNF) in mid-pregnancy and neonatal blood specimens as early biologic markers for autism.

Methods: We conducted a population-based case-control study nested within the cohort of infants born from July 2000 – September 2001 to women who participated in the prenatal screening program in Orange County, California. Cases (n=84) were all children receiving services for autism at the Regional Center of Orange County. Two comparison groups from the same study population were included: children with mental retardation or developmental delay (n=49) receiving services at the same regional center, and children not receiving services for developmental disabilities, randomly sampled from the California birth certificate files (n=159), and frequency-matched to autism cases on sex, birth year, and birth month. BDNF concentrations were measured in archived mid-pregnancy and neonatal blood specimens drawn during routine prenatal and newborn screening using a highly sensitive bead-based assay (Luminex).

Results: The concentration of BDNF in maternal mid-pregnancy and neonatal specimens was similar across all three study groups.

Corresponding author: Lisa A. Croen, PhD. Division of Research, Kaiser Permanente, 2000 Broadway, Oakland, CA 94612. Tel: (510) 891-3463; Fax: (510) 891-3802; Email: Lisa.A.Croen@kp.org.

Conclusion: These data do not support previous findings of an association between BDNF and autism and suggest that the concentration of BDNF during critical periods of early neurodevelopment is not likely to be a useful biomarker for autism susceptibility.

Keywords

biologic markers; neurotrophin; autism; BDNF; prenatal

Introduction

Autism Spectrum Disorders (ASD) are characterized by impairments in social interaction, verbal and nonverbal communication, and stereotyped behaviors and interests (Lord et al. 2000), and diagnosis is based solely on behavioral criteria (Willemsen-Swinkels and Buitelaar 2002). The etiology of ASD is not well understood, though it likely involves genetic, immunologic, and environmental factors (Volkmar et al. 2004). The dramatic increase in reported prevalence has spurred an intense effort to identify early biological markers (Chakrabarti and Fombonne 2005). Such markers could allow for earlier identification and therapeutic intervention, contributing to improved prognosis (Aman 2005).

Brain-derived neurotrophic factor (BDNF) is a small protein found throughout the central nervous system (CNS) and peripheral blood. BDNF is involved in the survival and differentiation of dopaminergic neurons in the developing brain (Hyman et al. 1991), and plays an important role in the formation and plasticity of synaptic connections (Binder and Scharfman 2004). BDNF is trophic for serotonergic neurons, and abnormalities in serotonin levels are the most common biochemical findings in autism (Anderson 2002;Tsai 2005). Animal studies suggest that concentrations of BDNF in the CNS and serum are closely correlated (Karege et al. 2002), offering the possibility that concentrations in peripheral blood may be useful as a possible biologic marker for autism.

Skewed expression of BDNF has been linked to neurologic and psychiatric disorders including Fragile X syndrome, epilepsy, Parkinson’s disease, Alzheimer’s disease, schizophrenia and depression (Pezet and Malcangio 2004;Binder and Scharfman 2004;Hashimoto et al. 2004;Angelucci et al. 2005). Elevated levels of BDNF were found in postmortem brain tissue from adults with autism (Perry et al. 2001) and concentrations different from those in controls have been found in peripheral blood of adults and children diagnosed with autism (Miyazaki et al. 2004;Connolly et al. 2006; Hashimoto et al. 2006;Enstrom et al. 2008). The two studies that have examined BDNF levels in neonatal specimens from individuals later diagnosed with autism have yielded inconsistent results (Nelson et al. 2001;Nelson et al. 2006).

In the present study we sought to examine the association between ASD and levels of BDNF in maternal mid-pregnancy serum and newborn blood specimens.

Methods

Subjects

The study sample was based on the Early Markers for Autism (EMA) Study, a population-based, nested case-control study designed to evaluate biologic markers of susceptibility and exposure in archived maternal mid-pregnancy and neonatal blood specimens from the same mother-baby pairs. The study population was drawn from the cohort of children born in California from July 2000 –September 2001 to women who were pregnant in Orange County, CA, and who participated in the State’s prenatal expanded alpha-fetoprotein screening program (XAFP). Only mother-child pairs for whom both a maternal mid-pregnancy and neonatal specimen was found in the archive (described below) were eligible for inclusion. All study procedures were approved by the institutional review boards of the California Health and Human Services Agency and Kaiser Permanente Northern California.

Three groups of children were identified: children with autism, children with mental retardation (MR) but not autism, and general population controls (GP). Children with autism or MR were ascertained from the California Department of Developmental Services (DDS), which operates a system of 21 Regional Centers (RC) that coordinate services for persons with autism, mental retardation, and other developmental disabilities. The system is widely utilized across socio-economic levels and racial/ethnic groups. DDS maintains a centralized electronic database of diagnostic and identifying information on all clients, derived from information provided by the RCs on a standardized, statewide reporting form. DDS and the RCs are mandated to provide services to individuals with autistic disorder and children with other Pervasive Developmental Disorders (PDDs) who have MR (IQ <70) or are substantially handicapped, as well as children with MR. GP controls were randomly sampled from the birth certificate files after excluding all past or current DDS/RC clients and were frequency matched to autism cases by sex, birth month and birth year at a 2:1 ratio.

Diagnostic verification

Following a protocol initially developed by the Metropolitan Atlanta Developmental Disabilities Surveillance Program (Yeargin-Allsopp et al. 2003), trained medical record abstractors reviewed and abstracted detailed diagnostic and clinical data from RC records for all subjects with autism or MR. Expert clinical review was conducted of abstracted data to classify children with autism by subtype based on DSM-IV-TR criteria, by onset type, and by cognitive status. Onset type was based on parental report or clinical observations and categorized as “early” (no statement of loss of social and/or language skills, or statement of early and sustained delays or plateauing of skills without actual loss), “regressive” (clear loss of previously acquired language and/or social skills), or unknown. MR determination was based on composite scores on standardized cognitive and functional tests (MR: composite score < 70; no MR: all scores > 70 or some scores <70 and others >70; unknown: no standardized scores in chart). The final analytic sample consisted of 84 children with autism (autistic disorder [DSM-IV-TR 299.0] (n=55), Asperger’s disorder [299.8] (n=0), PDD-NOS [299.8] (n=5), unknown (n=22); early onset (n=64), regressive (n=17), unknown (n=3); with MR (n=34), without MR (n=30), MR status unknown (n=20)); 49 children with MR but not autism (mild [DSM-IV-TR 317] (n=20), moderate [318.0] (n=12); severe [318.1] (n=11); profound [318.2] (n=3); unspecified [319] (n=3); known etiology (n=29), unknown etiology (n=20)); and 159 GP controls.

Specimen Collection

Maternal mid-pregnancy and neonatal blood specimens were retrieved from the prenatal and newborn screening specimen archives maintained by the California Department of Public Health. The prenatal specimen archive contains nearly 200,000 serum and blood cell pellet specimens remaining from women enrolled in the State’s XAFP screening program between November 1999 and December 2002 and living in three Southern California counties. As part of the screening program, venous blood was collected at 15–19 weeks gestation in serum separator tubes by obstetrical care service providers and underwent XAFP testing at a single regional laboratory, typically within seven days of collection (median time = 3 days). During transit via US Postal Service to the regional screening laboratory, no effort was made to control the temperature of the specimens. After testing, leftover specimens were kept under refrigeration for 1–2 days and then stored at −20°C.

The neonatal specimen archive contains dried bloodspots collected for screening purposes on nearly every infant born in California (~500,000 per year) since 1980. All newborn blood specimens for children included in this study were obtained by the heel-stick method, usually within 24–48 hours of birth. The blood specimens were collected at the nursery on a special S&S filter paper and allowed to dry at room temperature prior to transport to the regional laboratory for routine screening for metabolic and other disorders. During transport, usually by courier from the hospital to a regional screening laboratory, the temperature of the specimens was not controlled. Blood spots remaining after routine testing were catalogued and stored at −20°C.

BDNF measurements

We examined the concentration of BDNF in both maternal mid-pregnancy and neonatal specimens. For the neonatal determination, two 3mm punches of each neonatal bloodspot were placed in 96 well plates and eluted with 180μl of a buffer composed of 25ml of 10mM phosphate buffered saline with pH 7.4 and a protease inhibitor tablet (Roche Complete Protease Inhibitor Cocktail (Roche Applied Science, Indianapolis, IN)). The plates were then placed on a shaker for 1 hour at room temperature to thoroughly extract the blood sample from the paper spot. After incubation, eluates were transferred into cryogenic tubes and stored at −25°C until analyzed. Prior to analysis, serum and bloodspot eluates were thawed and centrifuged for 10 minutes.

BDNF concentration in undiluted bloodspot eluates and maternal serum was measured using a commercially available bead based Luminex kit (Biosource Human BDNF Antibody Bead Kit, Invitrogen-Biosource, Carlsbad, CA) in accordance with the kit protocols. Briefly, 50μl of sample was incubated with anti-BDNF-conjugated beads on a plate shaker for two hours, washed, and detection antibody was added for 1-hour incubation. Following washing, streptavidin-PE was added for 30 minutes. The plates were then read on a Bio-Plex 100 (BioRad, Hercules, CA), and analyzed using Bio-Plex Manager software with a 5-point curve.

Total protein for each bloodspot sample was measured with a commercially available kit (Pierce BCA protein assay, Pierce Biotechnology, Rockford, IL). The bloodspots were normalized for total protein concentration using the Bradford protein assay. This normalized for differences due to original spotting and elution. There was no need to normalize the maternal specimens for protein content as these were undiluted, whole blood serum. Internal standards were run to standardize the tests between plates.

Statistical Analysis

The distributions of BDNF levels in mother-baby pairs in the autism group were compared to the distributions in the GP and MR groups using two-sample *t*-tests (means) and Kruskal-Wallis tests (medians). To explore patterns of association between BDNF concentrations and autism risk, we compared categories of BDNF level [0–33% (reference), 34%-66%, 67%-90%, >90%)] across study groups with a chi-square statistic. Finally, to examine the association of autism with BDNF levels after adjustment for possible confounders, we fit separate logistic regression models for autism vs. GP controls and autism vs. MR controls. Autism vs. control status was regressed on $\log_{10}$-transformed BDNF levels with adjustment for several covariates related to the blood draw or associated with autism in previous epidemiologic studies. Separate models were run for maternal mid-pregnancy and neonatal specimens. Total protein was included as a covariate in neonatal specimen models to normalize the BDNF levels with respect to total protein concentration of the eluted bloodspot. For all newborn BDNF values that were below the limit of detection (LOD), we assigned a value of LOD/2; all maternal values were above the detection limit.

Results

Compared to the MR and GP control children, children with autism were significantly less likely to have a mother who was Hispanic or born in Mexico and more likely to have an older mother. Children with autism were significantly more likely than children with MR to be male and born in 2000 (Table 1).

Using Luminex technology and the same antibodies for maternal and newborn specimens, the concentration of BDNF was substantially higher (~100-fold) in maternal mid-pregnancy specimens than neonatal specimens (Table 2). BDNF levels in the two specimen types were not correlated (r=−0.15, P=0.08). Significant differences in mean BDNF concentrations, measured as either pg/ml or pg/ug, between the three diagnostic groups were not detected in either specimen type (Table 2). Comparisons of percentile categories of BDNF concentrations between autism vs. GP controls and autism vs. MR also failed to yield any significant differences for maternal or neonatal specimens (data not shown). BDNF concentrations in maternal and newborn specimens (BDNF measured as a continuous variable) were not associated with risk of autism in either crude or adjusted models (relative to the GP controls or MR controls) (Table 3). No differences in maternal or newborn BDNF levels were observed between autism subgroups (regression vs. early onset; autism with MR vs. autism without MR), or between autism subgroups and GP controls (data not shown).

Discussion

In our evaluation of BDNF concentrations in maternal mid-pregnancy and newborn specimens using Luminex technology, we found no differences between subjects with autism compared to subjects with MR or to general population controls. BDNF was initially identified as a possible biologic marker for autism in an early study using newborn blood specimens and a single antibody immunoassay (Nelson, et al. 2001). In that study, increased levels of BDNF were found in specimens from children with ASD or mental retardation compared to children with cerebral palsy or controls (Nelson et al. 2001). A subsequent study using both a Luminex double-antibody immunoaffinity bead-based assay and ELISA technology found no case-control differences in neonatal BDNF levels compared to typically developing control children (Nelson et al. 2006).

Evaluations of BDNF in older subjects have consistently found differences between those with autism and controls, with higher levels associated with autism in most studies. Miyazaki *et al* measured serum BDNF concentrations using commercial ELISA kits and found BDNF concentrations elevated in the ASD (mean age 7.6 years) and MR (mean age 11 years) groups compared to the controls (mean age 23.3 years); however, differences in age between study groups makes interpretation of these data difficult (Miyazaki et al. 2004). Also using commercial ELISA kits, Hashimoto *et al* reported significantly lower serum BDNF levels in autistic males (age 18–26) compared to age- and sex-matched healthy controls (Hashimoto et al. 2006). Connolly *et al* measured concentrations of BDNF and antibodies to BDNF in children with ASD, Landau-Kleffner Syndrome, Epilepsy, pervasive developmental disorder-not otherwise specified (PDD-NOS), childhood disintegrative disorder (CDD), healthy normal controls, and non-neurological illness (NNI) (Connolly et al. 2006). Using ELISA technology, BDNF levels were higher in children with ASD than healthy control and NNI children, but not significantly distinguishable from the other groups; higher titers of antibodies to BDNF were also found in children with ASD or CDD compared to healthy controls. Finally, using Luminex technology, Enstrom *et al* reported significantly elevated BDNF levels in the plasma of 2–5 year old children with autism compared to age-matched non-autistic controls (Enstrom et al. 2008). A trend toward higher BDNF levels in children with early onset autism compared to children with regressive autism was noted (studies summarized in Table 4).

Differences in the analytic platforms used, assay methods, and subject populations may explain the inconsistencies among studies. The antibodies used for capture and detection in the various assays may recognize different forms of the BDNF molecule and differences in dilution factors or developmental age of subjects at specimen collection are likely to influence results (Nelson et al. 2006;Katoh-Semba et al. 2007). The Luminex bead-based assay used for BDNF determinations in our study is highly sensitive (<10pg/mL), and serum levels in maternal mid-pregnancy specimens were similar to those reported previously (Lommatzsch et al. 2006), whereas the standard ELISA assay only measures down to the ng/ml range. If elevated BDNF levels characterize a specific autism endophenotype, inconsistent findings across studies may be related to differences in the phenotypic composition of the populations studied. As noted by Nelson, et al (2006), resolution of differences across studies would be aided by definition, using mass spectroscopy or a micro-chromatographic method, of the molecular nature of the material captured by different immunoassay methods.

In conclusion, BDNF concentrations in maternal mid-pregnancy and newborn blood specimens did not differentiate children with autism from control children. Larger studies adequately powered to investigate differences by autism phenotypes are needed.

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Table 1

Demographic Characteristics of Participants in the Early Markers for Autism (EMA) Study

	Autism (N=84)		GP Control (N=159)		MR Control (N=49)		Autism vs. GP control	Autism vs. MR control
	N	**(%)**	**N**	**(%)**	**N**	**(%)**	**P-value***	**P-value***
Gender							0.91	0.0003
Male	73	(86.9)	139	(87.4)	29	(59.2)		
Female	11	(13.1)	20	(12.6)	20	(40.8)		
Birth Year							0.16	0.003
2000	23	(27.4)	31	(19.5)	26	(53.1)		
2001	61	(72.6)	128	(80.5)	23	(46.9)		
Plurality							0.42	0.88
Singleton	81	(96.4)	156	(98.1)	47	(95.9)		
Multiple	3	(3.6)	7	(1.9)	2	(4.1)		
Mother's Race							0.17	0.76
White	57	(69.5)	126	(79.2)	37	(75.5)		
Asian	19	(23.2)	28	(17.6)	9	(18.4)		
Other	6	(7.3)	5	(3.1)	3	(6.1)		
Mother's Ethnicity							0.0007	<0.0001
Hispanic	20	(23.8)	73	(45.6)	28	(57.1)		
Non-Hispanic	64	(76.2)	86	(54.1)	21	(42.9)		
Mother's Birth Country							<0.0001	<0.0001
US	45	(53.6)	71	(44.7)	16	(32.7)		
Mexico	9	(10.7)	58	(36.5)	22	(44.9)		
Other	30	(35.7)	30	(18.9)	11	(22.4)		
Maternal Age (years)	30.9	(5.2)	28.2	(5.5)	28.3	(5.3)	0.0003	0.007
Maternal Weight at XAFP blood draw (lbs)	145.1	(26.7)	146.9	(33.8)	150.3	(39.3)	0.65	0.42
Gestational Age at XAFP blood draw (days)	119.8	(7.9)	118.8	(7.4)	118.4	(8.6)	0.35	0.36
Gestational Age at birth(days)	272.1	(18.5)	271.0	(14.3)	266.3	(28.1)	0.63	0.20
	Median	(25–75%)	Median	(25–75%)	Median	(25–75%)	P-value*	P-value*
Baby Age at NBS Blood Draw (days) Median	1.2	(1.0–1.8)	1.2	(1.0–1.6)	1.4	(1.1–2.2)	0.37	0.25

SD is standard deviation

*Based on chi-square test for categorical variables, two-sample T-test for means, Kruskal-Wallis for medians

Table 2

BDNF concentration measured by Luminex in mid-pregnancy serum and newborn dried bloodspot eluates from autistic subjects compared to general population (GP) and MR control subjects in the Early Markers for Autism (EMA) Study

	N	Mean	SD	P-value*	Median	Range	P-value*
Maternal Mid-Pregnancy BDNF (pg/ml)**							
Autism	34	3860.9	1345.7		3923.8	549.4-8468.9	
GP Control	78	3917.6	1312.7	0.83	4080.9	863.3-6881.9	0.57
MR Control	20	4337.6	1391.6	0.22	4350.2	1525.3-7416.3	0.12
Neonatal BDNF (pg/ml)							
Autism	84	54.1	40.0		54.4	6.1-187.0	
GP Control	159	53.7	47.7	0.95	49.5	6.1-309.1	0.53
MR Control	49	46.1	37.4	0.26	36.1	6.1-131.1	0.26
Neonatal BDNF/protein (pg/ug)***							
Autism	84	0.0083	0.0062		0.0081	0.0007-0.0275	
GP Control	159	0.0082	0.0073	0.91	0.0074	0.0005-0.0485	0.46
MR Control	49	0.0077	0.0068	0.57	0.0059	0.0006-0.0315	0.33

SD is standard deviation.

* All comparisons are to the autism group. Means compared with a two-sample t-test; medians compared with a Kruskal-Wallis test.

** BDNF assay run on only a random sample of maternal specimens.

Table 3

Risk of autism associated with a $\log_{10}$-unit increase in the concentration of BDNF measured in mid-pregnancy serum and newborn dried bloodspot eluates, Early Markers for Autism (EMA) Study.

	Autism vs. GP Control		**Autism vs. MR Control**	
	OR_{crude}(95% CI)	OR_{adj} (95% CI)	OR_{crude} (95% CI)	OR_{adj} (95% CI)
Prenatal BDNF	0.8 (0.28–2.1)	0.74[a] (0.22–2.5)	0.8 (0.29–2.1)	1.4[c] (0.19–10.7)
Neonatal BDNF	1.1 (0.83–1.4)	1.1[b] (0.78–1.7)	1.2 (0.85–1.6)	0.81[d] (0.40–1.6)

[a] Adjusted for maternal age, race, ethnicity, birth place, weight at prenatal blood draw, gestational age at prenatal blood draw

[b] Adjusted for maternal age, race, ethnicity, birth place, gestational age, baby's age at newborn screening, total protein, and plate

[c] Adjusted for maternal age, race, ethnicity, birth place, weight at prenatal blood draw, gestational age at prenatal blood draw, sex, birth year

[d] Adjusted for maternal age, race, ethnicity, birth place, gestational age, baby's age at newborn screening, total protein, plate, sex, birth year

Note: individuals with race=‘other’ or race=‘missing’ excluded from table

Table 4

Published studies on BDNF levels in peripheral blood and autism.

Reference (location)	ASD Group(s), Specimen Collection Age Range, Mean (SD)	Control Group(s), Specimen Collection Age Range, Mean (SD)	Specimen Type	Assay Method	BDNF Concentration Mean (SD)	Results
Nelson et al, 2001 (California)	**ASD:** n=69, Children born 1983–1985, 2.2 (1.2) days	**MR:** n=60, 1983–1985 CA births, 2.5 (1.7) days **CP:** n=63, 1983–1985 CA births, 5.4 (5.9) days **GP:** n=54, 1983–1985 CA births, 2.6 (1.6) days	Dried newborn bloodspots	Recycling Affinity Chromatography	**ASD:** 37.4 (19.9) pg/ml **MR:** 36.8 (18.6) pg/ml **CP:** 16.3 (9.7) pg/ml **GP:** 13.3 (5.0) pg/ml	Neonatal concentration significantly higher in ASD and MR groups compared to CP and GP controls; no difference between ASD and MR groups
Miyazaki et al, 2004 (Japan)	**ASD:** n=18, 3–27 years, 7.6 (6.1) years	**MR:** n=20, 1–20 years, 11.0 (5.9) years **HC:** n=16, 22–24 years, 23.3 (0.9) years	Serum	ELISA	**ASD:** 25.2 (2.5) ng/ml **MR:** 29.0 (2.3) ng/ml **Healthy:** 17.5 (2.0) ng/ml	Mean concentration higher in ASD and MR groups than HC: no difference between ASD and MR groups
Connolly et al, 2005 (Missouri)	**Autism:** n=37, 5.9 (3.9) years **CDD:** n=19, 7.0 (2.4) years **PDD-NOS:** n=14, 4.8 (3.9) years	**Landau Kleffner:** n=9, 7.4 (2.3) years **Epilepsy:** n=33, 5.9 (3.9) years **HC:** n=17, 4.3 (2.0) years **NNI:** n=16, 4.0 (3.2) years	Serum	ELISA	**Autism:** 32,279 pg/ml **CDD:** 36,872 pg/ml **HC:** 8,708 pg/ml **NNI:** 8,964 pg/ml	Mean concentration significantly higher in Autism and CDD than HC and NNI; Autism, CDD, and epilepsy had anti-BDNF IgG and IgM
Nelson et al, 2006 (California)	**ASD:** n=27, Children born in 1995, 1.2 (0.89) days	**DS:** n=26, 1995 CA births, 1.2 (0.9) days **GP:** n=20, 1995 CA births, 1.2 (0.9) days	Dried newborn bloodspots	Luminex	**ASD:** 3404 (1131) pg/ml, 27.4 (9.4) pg/mg **DS:** 3211 (1312) pg/ml, 23.1 (1.0) pg/mg **GP:** 3299 (844) pg/ml, 26.4 (8.2) pg/mg	No difference in mean concentration between groups
Hashimoto et al, 2006 (Japan)	**Autism:** n=18, 18–26 years, 21.2 (2.1) years	**HC:** n=18, 18–26 years, 22.2 (2.2) years	Serum	ELISA	**Autism:** 25.6 (2.2) ng/ml **HC:** 61.6 (10.9) ng/ml	Mean concentration significantly lower in Autism than HC group
Katoh- Semba et al, 2007 (Japan)	**Autism:** n=56, 0–59 years	**HC:** n=218, 0–59 years	Serum	Bead-based two- site sandwich enzyme immunoassay	0–9 year olds: **Autism:** 174.1 (190.4) pg/ml **HC:** 400.6 (148.8) pg/ml 10–19 year olds: **Autism:** 552.5 (354.3) pg/ml **HC:** 323.4 (118.8) pg/ml	Compared to HC group, mean concentration in Autism significantly lower in 0–9 year olds, and significantly higher in teenagers

Reference (location)	ASD Group(s), Specimen Collection Age Range, Mean (SD)	Control Group(s), Specimen Collection Age Range, Mean (SD)	Specimen Type	Assay Method	BDNF Concentration Mean (SD)	Results
Enstrom et al, 2008 (California)	**Autism:** n=37, 2.1–5.9 years, 3.58 years (median)	**DD:** n=6, 2.0–3.4 years, 2.71 years (median) **TD:** n=27, 2.2–4.6 years, 3.1 years (median) **SIB:** n=9, 2.3–4.6 years, 3.8 years (median)	Plasma	Luminex	**Autism:** 2512.1 (327.9) pg/ml **DD:** 1457.6 (211.1) pg/ml **TD:** 1463.1 (174.6) pg/ml **SIB:** 1444.7 (164.8) pg/ml	Mean concentration significantly higher in Autism compared to all control groups; early onset group higher than regressive group

ASD: autism spectrum disorder; **CDD:** childhood disintegrative disorder; **CP:** cerebral palsy; **PDD-NOS:** pervasive developmental disorder-not otherwise specified; **MR:** mental retardation without autism; **TD:** typically developing; **GP:** general population; **HC**: healthy controls; **SIB:** sibling of autism; **DD:** developmental delay; **NNI:** non-neurological illness; **DS:** Down syndrome